Table III—Determination of Impurity in Commercial Samples

Production Sample	Impurity Found, %[a]	Mean Deviation, %
A	3.60	±0.04
B	3.54	±0.02
C	1.76	±0.06
D	0.71	±0.07

[a] Average of triplicate assays.

the dipole moment, creating a more intensive absorption band due to the greater vibration. Electron-withdrawing groups such as halogens have the opposite effect. Table II lists the respective frequencies of acid carbonyl band absorption of 1% acetonitrile solutions of compounds with electron-withdrawing and electron-donating substitution *ortho* to the carboxylic acid group. These data indicate that both the presence of intramolecular hydrogen bonding and whether *ortho* substitution is electron-withdrawing or -donating, contribute to a 40–50-cm.$^{-1}$ frequency difference. Such differences in frequency make impurity determinations by the proposed method possible. Furthermore, it was observed that electron-donating and -withdrawing substitution *meta* to the carbonyl groups has minimal or no effect on the carbonyl band displacement.

The proposed method can be applied successfully to those samples where the contamination is 1% or greater (see Table III). Below this amount the accuracy of the method is limited; however, results obtained in such cases have shown agreement with those estimated by collaborative methods such as TLC and GLC.

SUMMARY

A new technique in the field of IR spectroscopy has been developed which offers the possibility of quantitative analysis of very weak absorption bands appearing as shoulders. These shoulders may often be resolved to measurable bands permitting accurate evaluations to be made in cases where previously reported techniques fail to work.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 9, 1969, from the *Analytical Research and Development Division of Ciba Pharmaceutical Company, Summit, NJ 07901*

Accepted for publication March 14, 1969.

Synthesis of Certain N^1- and N^4-(5-Nitro-2-pyridyl)-Substituted Sulfonamides

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Abstract □ The condensation of 2-chloro-5-nitropyridine with a number of sulfonamides is described. Sulfadiazine, sulfamerazine, sulfathiazole, sulfapyridine, sulfisoxazole, sulfamethoxypyridazine, and sulfadimethoxine afforded the N^4 derivatives *via* the application of Banks' procedure. 4-Homosulfanilamide using Ullmann's and Mangini's conditions, yielded the N^1 and N^4 derivatives, respectively. With benzylsulfonamide and benzenesulfonamide adopting Ullmann's method the corresponding *N*-substituted derivatives were obtained.

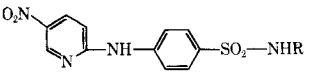
Keyphrases □ Sulfonamides, N^1, N^4-(5-nitro-2-pyridyl)-substituted —synthesis □ Condensation reaction—sulfonamide derivative synthesis

In two earlier publications (1, 2) the authors reported the condensation of 2-chloro-5-nitropyridine with different amino compounds including sulfaguanidine as a representative of the group of sulfonamides. In continuation, other sulfonamides were condensed with the same halonitroheterocycle, in the hope that the introduction of the 5-nitro-2-pyridyl moiety might enhance the antimicrobial activity of the parent compounds (3–7).

2-Chloro-5-nitropyridine was prepared from 2-aminopyridine according to a method described earlier (1). Of the sulfonamides: 4-homosulfanilamide and benzylsulfonamide were synthesized adopting reported procedures. The former compound was prepared from benzylamine by acetylation (8–11), chlorosulfonation, amidation of the sulfonyl chloride, subsequent hydrolysis of the acetylamino group, and finally isolating the product as the hydrochloride (12, 13). Benzyl sulfonamide was obtained from benzyl chloride by treatment with sodium sulfite, converting the sulfonate thus obtained to the acid chloride using phosphorus pentachloride and finally to the amide by mixing with excess concentrated ammonia (14).

Several attempts were made to condense 2-chloro-5-nitropyridine with the N^1-substituted sulfonamides. Bobranski's method (15, 16) did not give satisfactory results while Ullmann's conditions (17–19) were unsuccessful. Likewise, the use of solvents such as pyridine (20), pyridine-ethanol, dioxan, and ethylene glycol (in presence and absence of potassium carbonate) did not improve matters. Nevertheless, the condensation was successfully achieved by conducting it in aqueous hydrochloric acid in presence of varying amounts of ethanol (to enhance solubility and decrease volatility of 2-chloro-5-nitropyridine) following Banks' directions

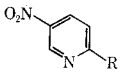
Table I—N^4-Substituted Derivatives of Some Sulfonamides



Compound	R	Reflux Time, hr.	Yield, %	Solvent of Crystallization[a]	Mp., °C.[b]	Formula	Anal.,[c] % Calcd.	Found
I	4-Methyl-2-pyrimidinyl	1	67	A	Above 300	$C_{16}H_{14}N_6O_4S$	C, 49.99 H, 4.15 N, 20.60	C, 50.33 H, 4.51 N, 21.11
II	2-Pyrimidinyl	3.5	67	A	Above 300	$C_{15}H_{12}N_6O_4S$	C, 48.35 H, 3.90 N, 20.60	C, 48.57 H, 3.77 N, 20.26
III	2-Thiazolyl	4.5	67	A	275–277	$C_{14}H_{11}N_5O_4S_2$	N, 16.50	N, 16.21
IV	6-Methoxy-3-pyridazinyl	2.5	70	A	Above 300	$C_{16}H_{14}N_6O_5S$	N, 18.70	N, 18.16
V	2-Pyridyl	4.5	67	A	248–250	$C_{16}H_{13}N_5O_4S$	N, 16.76	N, 16.67
VI	3,4 Dimethyl-5-isoxazolyl	30	67	B	205	$C_{14}H_{15}N_5O_5S$	C, 49.10 H, 3.85 N, 17.90	C, 49.96 H, 4.15 N, 17.45
VII	4,6-Dimethoxy-2-pyrimidinyl	10	66	B	210–212	$C_{17}H_{16}N_6O_6S$	C, 45.90 H, 4.80 N, 16.99	C, 45.65 H, 3.86 N, 17.01

[a] A, 95% ethanol, B, aqueous ethanol. [b] Melting points were performed by the capillary tube method and are uncorrected. [c] Analyses performed by El-Nasr Co. for Chemicals, U.A.R.

Table II—N^1-Substituted Derivatives of Some Sulfonamides



Compound	R	Fusion Time, min.	Yield, %	Solvent of Crystallization[a]	M.p., °C[b]	Formula	Anal.,[c] % Calcd.	Found
I	*p*-Acetylaminomethylbenzenesulfonamido	60	60	A	210	$C_{14}H_{14}N_4O_5S$	N, 16.00	N, 15.54
II	Benzylsulfonamido	20	53	B	260	$C_{12}H_{11}N_3O_4S$	N, 12.34	N, 11.76
III	Benzenesulfonamido	10	50	B	256–258	$C_{11}H_9N_3O_4S$	C, 47.68 H, 3.90 N, 13.90	C, 47.86 H, 4.36 N, 14.26

[a] A, aqueous ethanol, B 95% ethanol. [b] Melting points were performed by the capillary tube method and are uncorrected. [c] Analyses performed by El-Nasr Co. for Chemicals, U.A.R.

(21). That the condensation had taken place at the N^4 position was shown qualitatively by the failure of all the condensation products to diazotize.

Condensation of 2-chloro-5-nitropyridine with 4-homosulfanilamide afforded either the N^4 or N^1 derivative according to conditions. Applying Mangini's method (22) which consists in refluxing the reactants with absolute alcohol in presence of fused sodium acetate yielded the N^4 derivative. The same derivative was also obtained when the sodium acetate was replaced with one or two equivalents of sodium hydrogen carbonate. Applying Ullmann's conditions to N^4 acetyl homosulfanilamide, on the other hand, afforded the N^1-substituted derivative.

The *N*-substituted derivative of benzylsulfonamide was prepared *via* two different routes: condensation of 2-chloro-5-nitropyridine with benzylsulfonamide according to Ullmann's conditions or alternatively by reacting the potassium salt of the sulfa compound with the halonitroheterocycle in presence of dimethylformamide; which affords a higher yield of the condensation product.

With benzene sulfonamide, Ullmann's conditions (17–19) only were applied, and a complete reaction was attained after fusing the reactants for 10 min. However, with benzylsulfonamide, 20 min. were required.

EXPERIMENTAL[1]

N^4-Substituted Derivatives of Some Sulfonamides—*General Procedure*—Compounds I–VII were prepared by refluxing a mixture of equimolecular amounts of 2-chloro-5-nitropyridine and the sulfonamide with hydrochloric acid solution (6 ml. concentrated HCl, d. 1.19, in 19 ml. of water and 5 ml. of ethanol) for 1–30 hr. (according to the sulfa compound). The product separating from the reaction mixture was filtered, and washed with water until the washings gave a negative chloride test with silver nitrate T.S. The product after being dried was crystallized from the appropriate solvent (see Table I).

N^1-Substituted Derivatives of Some Sulfonamides—*General Procedure*—Compounds I–III were prepared by fusing a mixture of equimolecular amounts of 2-chloro-5-nitropyridine, the sulfonamide, anhydrous potassium carbonate, and a trace of copper powder at 160–180° for 10–60 min. (according to the sulfa compound). The solid cake resulting was then extracted with 50% aqueous ethanol. The deep red extract was acidified with 12% acetic acid. The product which was obtained was washed, dried, and crystallized from the appropriate solvent (see Table II).

[1] The compounds synthesized have been submitted for a general pharmacological screening and the results obtained will be the subject of a subsequent communication.

N⁴-(5-Nitro-2-pyridyl)-4-homosulfanilamide—A mixture of 2-chloro-5-nitropyridine (0.25 g.), 4-homosulfanilamide hydrochloride (0.32 g.), and freshly fused sodium acetate (0.2 g.) was refluxed with 30 ml. absolute ethanol for 24 hr., after which the reaction mixture was diluted with water until complete separation. The product which was obtained in 60% yield (0.3 g.) melted at 190° after being crystallized from ethanol.

Anal.—Calcd. for $C_{12}H_{12}N_4O_4S$: N, 18.30. Found: N, 17.97.

N-(5-Nitro-2-pyridyl)-benzylsulfonamide—A mixture of 2-chloro-5-nitropyridine (0.8 g.), potassium benzyl sulfonamide (0.9 g.) and dimethyl formamide (30 ml.) was heated at 110–130° for 3 hr. The reaction mixture was then evaporated under diminished pressure nearly to dryness, and the residue (1 g., 62% yield) was crystallized from ethanol. It melted at 260° and the melting point was not depressed on admixture with a pure specimen prepared by Ullmann's method.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 28, 1968 from the Organic Chemistry Department, Faculty of Pharmacy, Cairo University, United Arab Republic

Accepted for publication April 7, 1969.

COMMUNICATIONS

Simple Assay for Determination of Carbonic Anhydrase Activity

Keyphrases ☐ Carbonic anhydrase activity—analysis ☐ Carbon dioxide-^{14}C liberation, *in vitro*—carbonic anhydrase ☐ Scintillometry, liquid—radioactivity determination

Sir:

A method has been developed for the *in vitro* determination of carbonic anhydrase activity. The method is based on the principle that the reactions involved in attaining equilibrium between bicarbonate ion and carbon dioxide in solution are both catalyzed by carbonic anhydrase. Since the function of carbonic anhydrase *in vivo* is to maintain this equilibrium, the activity of the enzyme can be determined *in vitro* by shifting the equilibrium in the desired direction. Carbon dioxide-^{14}C liberated from labeled bicarbonate in a buffer system, containing carbonic anhydrase, is trapped on alkali-moistened filter paper strips. The radioactivity collected on the strips is subsequently determined by liquid scintillation counting. A preliminary report of this work has been presented (1).

Figure 1 illustrates the absorption tube used for collecting the labeled CO_2. A 1.59 × 3.81-cm. ($^5/_8$ × $1^1/_2$-in.) strip of Whatman No. 1 filter paper is impaled lengthwise on the wire of a tube stopper, and wetted with about 100 μl. of 0.5 *M* NaOH. The incubation mixture used consists of 1.2 ml. of phosphate buffer (two parts 0.1 *M* KH_2PO_4 and three parts 0.1 *M* Na_2HPO_4), pH 7.0, 0.2 ml. of enzyme preparation, and 1.0 ml. of $NaH^{14}CO_3$ solution (6.25×10^{-3} *M* containing 1.55 μc./m*M*). The substrate solution is dissolved in water and stored frozen. The enzyme preparations employed are listed below at the concentration routinely used and the useful range over which linearity is known to exist is recorded in parentheses, (*a*) 0.35 (0.07 to 0.70)% saline solutions of hemolyzed rat or dog

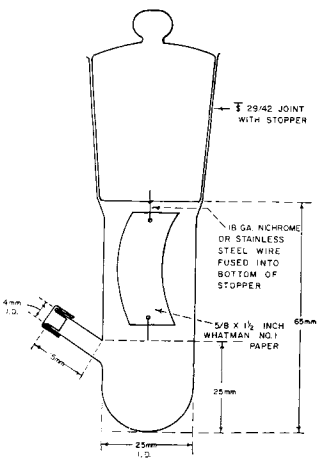

Figure 1—*Absorption tube used for collecting the labeled carbon dioxide.*